STUDIORUM PROGRESSUS

Brain Hormone Contained in the Silkworm Body of *Bombyx mori*

Introduction. Since KOPEĆ[1] first suggested the endocrine function of the insect brain in the gypsy moth, *Lymantria dispar*, and WIGGLESWORTH[2] demonstrated the initiation of moulting by a hormonal factor originating in the dorsal region of the protocerebrum in the bug, *Rhodnius prolixus*, the critical role of the cerebral neurosecretory system in moulting has been consistently verified. The thoracotropic action of the brain hormone (BH) was clearly demonstrated by WILLIAMS[3,4] in a giant silkmoth, *Hyalophora cecropia*. Since then, the BH was called thoracotrophic hormone or ecdysiotropin.

Although the brain hormone still resists being isolated, because of difficulties in obtaining a large amount of the starting materials and in developing a handy bioassay, it is thought to be a protein or polypeptide hormone[5–9] in accordance with what is known of neurosecretory hormone in other animals.

Many implantation and extirpation experiments or histological observations have demonstrated that the ecdysiotropin is vigorously produced in the cerebral neurosecretory cells throughout the entire active life cycle even after the thoracic glands have degenerated. ISHIZAKI[10] was the only man who checked the brain hormone titer chemically. He extracted and partially purified BH contained in the brain, head and thorax-abdomen of silkworms and determined the hormone titer by injection of the extracts into debrained pupae of *Samia*. BH titers in the brain and head of both sexes maintained similarly low activity in developing adults and then markedly increased shortly before adult emergence; this high activity lasted throughout adult life. Moreover, a striking accumulation of BH was found in the body of developing female adults and of newly emerged moths of some races but not in that of males of any race.

The following questions arose: 1. Is BH produced in the neurosecretory cells not liberated into the blood after degeneration of the prothoracic gland? 2. When is the BH found in the bodies of developing female adults and moths accumulated? And what is the meaning of BH accumulation when BH as an ecdysiotropin is apparently no longer necessary? 3. Is there any definite difference of BH titer among the races?

The present paper deals with the comparison of BH titers contained in the head and thorax-abdomen of both sexes of 3 races of silkworms during and post imaginal differentiation and also with the effect of debraining and castration on the BH titer in the body.

Materials and methods. Silkworm, *Bombyx mori*, was used as the source of BH.

For experiment on the racial and sexual differences of BH titer, silkworms from 3 representative races of Gunka (Japanese original strain), Hōshun (Chinese original strain) and a racial hybrid between Hōshun and Gunka were picked and reared on fresh mulberry leaves during the whole larval life (by courtesy of Gunze & Co., Ltd.). Males and females of each races were divided into 4 stages; P1 = pupa on the onset of imaginal differentiation (2–3 days after pupation at 25 °C), P2 = developing adult (6–7 days after pupation, whole pupal period was 9 days), AV = virgin adult within 12 h after emergence, and AM = adult after mating for 1 day.

All specimens were first frozen, having had removed antennae, legs and wings (P2, AV, AM), and head was separated from the thorax-abdomen with a sharp razor blade. We did not check the BH titer in the brain because 1. this organ is so small that technical errors during extraction and purification would be unavoidable and 2. there was found no lethal difference of BH titer between in the brain and head in ISHIZAKI's experiment[10].

For experiments on removal of the brain and castration, a hybrid of Shinki and Ryōhō, KC-KN (by courtesy of Shinei & Co., Ltd.) was reared on an artificial diet during its whole larval life. Within 12 h after emergence, all virgin adults were frozen. Only their thorax-abdomens were used to determine the BH titer.

Operation procedure. Removal of the brain or the suboesophageal ganglion was performed within 4–20 h after pupation under anesthesia by chilling and the wound was coated with melted paraffin.

Castration was performed on the 3rd day of the 4th instar larva without anesthesia and the wound was left intact after the 'blood' had been wiped off.

Sham operations on the controls were done, making corresponding wounds without removing the organs.

Extraction of BH. The extraction procedure was reported in the previous paper[9]. Acetone dried powder of each material was extracted with 20 times the volume of 2% NaCl, and the supernatant after heat treatment (90 °C, 3 min) was employed as a BH preparation.

Bioassay of BH. Samia test was performed to determine the hormone titer[9]. Each 5–10 pupae of *Samia cynthia-ricini* after the removal of their brains received 40 µl of

Table I. BH titer in the head and thorax-abdomen of 3 different races[a]

Stage[b]	Race[c]	*Samia* unit/individual[d]			
		Head		Thorax-abdomen	
		♀	♂	♀	♂
Pupa young	1	252	36	891	73
	2	252	36	1227	772
	3	252	288	996	214
Pupa aged	1	252	72	786	69
	2	—	72	—	175
	3	63	90	306	28
Adult virgin	1	158	245	721	0
	2	40	32	1121	0
	3	288	90	548	0
Adult mated	1	113	360	159	0
	2	242	147	—	0
	3	235	245	101	0

[a] For each experiment, 30–52 specimens (mainly 50) were employed. [b] See text. [c] 1. Japanese original race, Gunka. 2. Chinese original race, Hōshun. 3. Hōshun × Gunka. [d] Amount of BH obtained is expressed on an individual basis.

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the test solution. Brain hormone titer was measured by assaying a series of 2–4 fold diluted solutions of the preparation. Bioassays were performed as double tests with different test animals reared at different times.

Results. 1. Racial and sexual differences of BH titer. As has been pointed out by ISHIZAKI[10], racial and sexual differences of the BH titer were observed. Table I gives the BH titer among 3 races and sexes during and post imaginal differentiation.

Generally speaking, the BH titer in the female head was highest in the P1 stage, thereafter it decreased through the AV stage. A slight increase was again observed after mating (AM). The amount of BH detected in the male head was less than that in the female head. The BH activity, however, markedly increased in the adult male head after mating. These results were different from ISHIZAKI's in the amount of BH – our Samia unit/individual was much higher than his – as well as the stage which showed the maximum level of hormone activity – ours was P1, his AV.

The BH titer in the thorax-abdomen of females was also highest in the P1 stage, regardless of race. This result also differs from ISHIZAKI's in which the highest titer of BH was found in the newly emerged adult. The BH titer of the thorax-abdomen was 3–5 times higher than that of the head of the corresponding stages. Thereafter, it gradually decreased through the AV stage and markedly diminished after mating. Although ISHIZAKI could not detect any significant activity of BH in the male thorax-abdomen throughout larval to adult stages, the present result indicates that male pupae of all 3 races contained a considerable amount of BH in their thorax-abdomen even if the titer was $^1/_3$ of that of the female. The BH activity was not detected after emergence.

The average value of BH contained in the whole body indicates that the highest activity was in the young pupa (P1) of both sexes. However, females had 3 times as much hormone as males. Thereafter, BH activity decreased by stages. In females after mating (AM), the BH titer was to $^1/_4$ of that of young pupae. In the case of males, the tendency of BH titer decrease was the same as that of females but BH titer increased again after mating. The value for the whole body of adult males was the same as the BH of the head portion, because no BH was found in the thorax-abdomen.

2. BH titer after removal of the brain. If the BH found in the head is regarded as the BH contained in the brain, the neurosecretory cells of the brain seemed to be laden with secretion of the BH constantly as was observed in the previous experiment. We assumed, however, that the liberation of BH from the brain is another matter. When is the BH released from the brain and stored in the pupal and adult thorax-abdomen? Is the suboesophageal ganglion (SG) involved in this matter? To answer these questions, the brain (-Br) or the suboesophageal ganglion (-SG) was removed from the pupa within 4–20 h after pupation. Then the thorax-abdomen was examined for the BH titer within 12 h after emergence. The experiment was repeated twice in different seasons. Table II shows the sum of both results because of no difference of value between the two experiments.

Table II. BH titer in the adult thorax-abdomen of debrained and castrated silkworms

Sex	Operation procedure[a]	No. of specimens	Samia unit per individual[b]
♀	-Br.	104	314
	-SG	96	315
	Sham	96	287
♀	-Br.	77	0
	-SG	117	0
	Sham	98	0
♀	-Ovaries	56	1048
	Sham	56	312
	-Testes	51	0
♀	Sham	52	0

[a] -Br. or -SG: Brain or suboesophageal ganglion was removed within 20 h after pupatin. Castration was performed on the 3rd day of 4th instar larva. [b] Amount of BH obtained is expressed on an individual basis.

As seen in Table II, after removal of either brain or suboesophageal ganglion, the BH titer in the thorax-abdomen of the female adult showed exactly the same value as that found in the wound-only sham control. In the case of males, neither -Br nor -SG showed any BH activity like that of the control. These facts suggest that BH is excessively released from the brain into the body before or shortly after the pupation and the pupal brain is only laden with BH (see Table I) without mechanism required for liberation of the hormone. The suboesophageal ganglion was not involved in either BH accumulation in the body or consumption of BH of the body.

Note here that the *Samia* unit per individual female in Table II was only about 1/2 (compared with race 3) or 1/4 (compared with race 2) of that in the same AV stage of Table I. Generally, silkworms reared on artificial diets differ from those reared on fresh leaves in physiological and hormonal conditions. A marked reduction of BH titer was assumed to have resulted from the rearing on an artificial diet. The same phenomenon was also observed in the case of the castration sham control.

In most races of *Bombyx*, the critical period of BH secretion for imaginal development is in the prepupal period. According to KOBAYASHI[11], however, appearance of a high ratio of dormant pupa of *Bombyx mori* following removal of the brain shortly after pupation was only expected in special races, such as a racial hybrid of Japanese No. 122 and Chinese No. 115. In the present experiment, the race used was not a special one; moreover removal of the brain was performed within 4–20 h after pupation, when many hours had passed beyond the critical period of BH for emergence. Nevertheless, the so-called «dauer pupa» appeared in 4 females out of 100 which had been operated on and 30 males out of 97, 40 days post operation. The diapausing state continued in 28 male pupae over 4 months, and in 9 over 7 months. On the other hand, all specimens of sham-operated pupae emerged within 2 weeks (mainly 10 days) without exception. This fact also suggests the different hormonal conditions of worms reared on artificial diet from those reared on fresh leaves.

3. BH titer after castration. Since the amounts of BH detected in the pupal and adult bodies were markedly different between females and males, the possibility that the gonad might be involved was investigated. The castration operation was done on the 3rd day of the 4th instar larva and the BH titer of each sex was measured only with the thorax-abdomen of the newly emerged moth. Operation and bioassay were repeated twice and no difference between the two was found.

As indicated in Table II, castrated female adults showed hormone titers 3 times higher than those of the sham control females, although nothing was found in the bodies

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of both castrated and non-castrated male adults. These facts indicate that part of the BH accumulated in the female pupal body may be consumed for egg maturation and/or yolk deposition.

Discussion. Generally speaking, the amount of BH we obtained was much higher than ISHIZAKI's[10]. Besides his races employed were different from ours, and he dialyzed the BH preparations. As nearly $^1/_3$ of the active fractions of BH would escape from the cellulose tubing after dialyzation[9], we did not dialyze our BH preparations. These are some reasons why our BH titer was generally much higher than his.

The present experiments showed that the amount of BH in the thorax-abdomen was highest in the young pupae regardless of races. Thereafter it gradually decreased through the newly emerged adult stage and markedly diminished after mating. On the other hand, according to ISHIZAKI[10] the striking increase of BH quantity appears only in the females thorax-abdomen during adult development and reached a maximum level at the time of adult emergence. Although our silkworms were of different races from his, it seems unlikely that liberation of BH from the brain into the body continues during the pupal period. It is quite probable that shortly after the pupation, liberation of both BH and ecdysone[12] from the endocrine organs reach a maximum level, and thereafter, prothoracic glands degenerate and the brain maintains its secretory activity in the neurosecretory cells without liberation of BH until emergence. This hypothesis is also supported by the result of the debraining experiment in which the BH titer of moths newly emerged from the debrained pupae was exactly the same as the normal control.

Neurohormone from the brain appears directly or indirectly to control the egg maturation in some insects[13]. This is also supported by the present castration experiment in which the large amount of BH accumulating in the young female pupal body was consumed by the egg maturation and/or yolk deposition processes.

Male pupae had a considerable amount of BH in their bodies even if the titer was $^1/_3$ of the female one and activity of BH disappeared at the time of emergence. This fact may suggest that some BH is consumed during the adult development of both males and females.

The hormone(s) secreted in the neurosecretory cells of the brain is said to have a multiplicity of effects, such as moult inhibiting[14], tanning[15], and melanotropic[16] effects, besides prothoracotrophic and gonadotropic ones. However, whether the so-called 'Brain Hormone' is a hormone having various hormonal actions, or is a hormone complex, remains unanswered. At present, it seems hardly possible make any definite conclusions because no hormone has yet been isolated. The facts that the brains of developing adults secrete (or at least reserve) a hormone having ecdysiotropic action after the prothoracic glands have degenerated and a hormone having the same ecdysiotropic activity is liberated into and accumulated in the body before or just after pupation and consumed during egg development and copulation, suggest that BH at least as the thoracotrophic hormone has diversified actions.

In adult lepidoptera, juvenile hormone activity is higher in the male body[17] and ecdysone activity[12] as BH is higher in the female body; these hormones have no apparent hormonal roles in the adult stage. At present, we can only suggest, without clear proof, what is actually happening within the insect body.

Summary. The present report is concerned with the comparison of the brain hormone titers contained in the head and thorax-abdomen of both sexes of 3 races of silkworms, *Bombyx mori* during and post imaginal differentiation and also with the effects of debraining and castration on the brain hormone titer in the body.

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PRO EXPERIMENTIS

A Bipolar Stimulation Electrode for in vitro Stimulation of Small Pieces of Electric Organ of *Torpedo marmorata*

An increasing number of biological studies require qualitative and quantitative measurements of changes of vesicles in cholinergic synapses of *Torpedo marmorata* with reference to their density and diameter under the influence of drugs. To find out whether the number and size of vesicles in stimulated and unstimulated organs differ, the main nerves of the electric lobe were stimulated with repeated trains (DUNANT[1], NAEF[2]). As it is not possible to apply the drugs into the blood-circulatory system, they were poured directly into the electric organ. This method needs a large amount of drug solution and it cannot be assumed with certainty that the organ is throughly soaked with it.

In this short communication, an electrode system for direct stimulation and signal detection is described. With this system, it is possible to stimulate in vitro small (4 mm^3) as well as bigger (1 cm^3) pieces of electric organ of *Torpedo marmorata* (Figure 1).

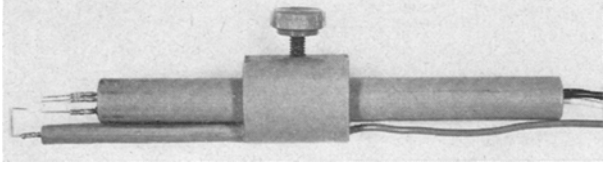

Fig. 1. Photograph of the electrode. ×0.5

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